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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

APR 16 1992

MEMORANDUM

SUBJECT: Tebuthiuron

HED Project No.: 1-2340
TOX Chem No.: 366AA

FROM: Ray Landolt 4/10/92
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

TO: Walter Waldrop, Product Manager 71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Mike Ioannou, Section Head
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Branch Chief
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

M. Ioannou 4/10/92

Marcia van Gemert 4/13/92

Registrant : Elanco Products Company

Action Requested: Review two mutagenicity studies.

1. In Vivo Sister Chromatid Exchange Assay in Chinese Hamster Bone Marrow. Study No. 880511SCE655, MRID No. 407509-02.

2. In vitro Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes Study No. 880510UDS0655 and 880517UDS0655, MRID 407509-01.

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Conclusion:

1. Under the condutions of the in vivo sister chromatid exchange (SCE) assay conducted with female Chinese hamsters (3/group), the single oral administration of 3000, 4000, or 5000 mg/kg tebuthiuron did not cause a significant increase in the frequency of SCEs.

   This study is deficient for the number of animals tested, three per dose rather than the recommended five per dose.

   Classification of Data: Unacceptable

2. Under the conditions of two independently performed unscheduled DNA synthesis (UDS) assays, at concentrations ranging from 300 to 800 ug/mL, tebuthiuron did not induce UDS in primary rat hepatocytes.

   This study is deficient due to the number of cells per culture counted, 20 cells per culture rather than the recommended 50 cells per culture. This study may be upgraded if the slides are available and the data from the analysis of at least 50 cells per culture are submitted for review.

   Classification of Data: Unacceptable

DOC 920079
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DATA EVALUATION REPORT

TEBUTHIURON

Study Type: Mutagenicity: In Vivo Sister Chromatid Exchange Assay in Chinese Hamster Bone Marrow

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne T. Haber Ph.D. Date 4/7/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 4/7/92

QA/QC Manager Sharon Segal, Ph.D. Date 4/7/92

Contract Number: 68D10075
Work Assignment Number: 1-30
Clement Number: 91-96
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY IN VIVO SCE

MUTAGENICITY STUDIES

EPA Reviewer: Ray Landolt Signature: Ray Landolt
Review Section I, Toxicology Branch (II)/HED Date: 4/8/1992
EPA Section Head: Yiannakis Ioannou, Ph.D. Signature: Yiannakis Ioannou
Review Section I, Toxicology Branch (II)/HED Date: 4/9/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo sister chromatid exchange assay in Chinese hamsters.

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 366AA

MRID Number: 407509-02

TEST MATERIAL: Tebuthiuron

SYNONYMS: N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea

SPONSOR: Eli Lilly and Company, Greenfield, Indiana

STUDY NUMBER: 880511SCE655

TESTING FACILITY: Eli Lilly and Company, Greenfield, Indiana

TITLE OF REPORT: The Effect of Tebuthiuron (EL-103, Compound 075503) on the In Vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters.

AUTHORS: Negilski, D. S., Garriott, M.L., and Brunny, J. D.

REPORT ISSUED: July 13, 1988

CONCLUSIONS-EXECUTIVE SUMMARY: Under the conditions of the in vivo sister chromatid exchange (SCE) assay conducted with female Chinese hamsters (3/group), the single oral administration of 3000, 4000, or 5000 mg/kg tebuthiuron did not cause a significant increase in the frequency of SCEs. Compound toxicity (hypoactivity) was seen in all treatment groups and clear evidence of cytotoxic effects on the target organ (bone marrow cells) was observed in the 5000 mg/kg group. These findings indicated that the test material was evaluated to the maximum tolerated dose. However, fewer animals (3 females) than recommended by Guideline (5 females and 5 males) were tested. In addition, the report did not specify the procedures used to assign the animals to treatment and control groups, and whether slides were coded prior to analysis. Thus, the study does not fully satisfy Guideline requirements for genetic effects Category III, Other Mutagenic Mechanisms.

STUDY CLASSIFICATION: The study is unacceptable.

A. MATERIALS:

1. Test Material: Tebuthiuron

Description: None provided
Identification No: Compound 075503; Lot no.: 729AS7
Purity: 99.1%
Receipt date: Not reported
Stability: Not reported
Contaminants: None listed
Solvent used: 10% aqueous acacia
Other provided information: Neither storage conditions nor the frequency of dosing solution preparation were reported. Doses for the preliminary toxicity study were prepared on the day administered.

2. Control Materials:

Vehicle/final concentration/route of administration: 10% aqueous acacia (10mL/kg) was administered orally.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was orally administered at 50 mg/kg in 10% aqueous acacia.

3. Test Compound:

Route of administration: Oral

Dose levels used:

(a) Preliminary toxicity assay: 1000, 2000, 3000, 4000, and 5000 mg/kg (5 females per dose)

(b) SCE assay: 3000, 4000, and 5000 mg/kg (3 females per dose)

4. Test Animals: Female Chinese hamsters, Cricetulus griseus, were bred and maintained at the reporting laboratory. They were used at least two weeks after reaching adulthood. Animals used for the preliminary toxicity assay weighed 30-44 g, and those used for the SCE assay weighed 32-39 g. Animals were housed individually in an environment controlled for temperature (24 ±3°C), humidity (≥40%), and light (12 hours).

B. STUDY DESIGN:

1. Preliminary Toxicity Assay: Groups of five 4-7-month-old females were administered the selected test material doses or vehicle. The animals were observed frequently for the first few hours following treatment and daily thereafter, for a total of 48 hours.

2. SCE Assay:

a. Test compound administration: Based on the results of the preliminary toxicity assay, three doses of the test compound were examined in the SCE assay. Three female hamsters per dose received single oral administrations of the test compound, vehicle, or positive control at a dosing volume of ≤10 mL/kg. The report did not indicate the method used to assign the animals to treatment and control groups.

b. BrdU implantation/Velban® administration/animal sacrifice: Five hours prior to administration of the test compound, vehicle, or positive control, a 20-30 mg agar-coated BrdU tablet was subcutaneously implanted in the abdomen of each ether-anesthetized animal. At 19 hours posttreatment, Velban® (1 mg/kg) was injected intraperitoneally to arrest the cells in metaphase, and the animals were sacrificed two hours later by ether narcosis.

c. Slide preparation: Bone marrow cells were flushed from femurs with 0.075 M KCl, centrifuged, washed and fixed in methanol-acetic acid (3:1). Metaphase preparations were made by standard techniques. Slides containing metaphase chromosomes were maintained in the dark for at least 24 hours before staining with Hoechst 33258. Slides were sealed with lacquer, exposed to 366 nm UV light, washed, and stained in a 3% aqueous solution of Giemsa.

d. Slide analysis: Twenty-five well-spread representative metaphases, each containing the diploid number of chromosomes and demonstrating second division sister chromatid differentiation, were scored for SCE in each animal. The report did not indicate whether slides were coded prior to analysis.

e. Cytotoxicity analysis: A total of 100 metaphase figures per animal were scored for first, second, or third division staining. An increase in the number of first division figures was interpreted as an indication of cytotoxicity.

3. Evaluation Criteria: The assay was considered positive if at least two successive concentrations of the compound resulted in SCE frequencies significantly higher than the vehicle control. To determine statistical significance, the variance of the mean SCE values of individual animals was calculated, and group means were compared according to Dunnett[1]. Defining significance as $p \leq 0.01$,

[1]Dunnett, C.W., (1964). New tables for multiple comparison with a control. Biometrics 20:482.

an increase of 1.1 to 1.5 SCE/metaphase over the control was considered significant using this method. The positive control was compared to the negative control using Student's t-test, with significance defined as $p \leq 0.01$.

5. Protocol: See Appendix B.

C. REPORTED RESULTS:

1. Preliminary Toxicity Assay: Five levels of tebuthiuron, ranging from 1000 to 5000 mg/kg, in 1000 mg/kg increments, were orally administered to five females per dose. No adverse effects were observed at 1000 mg/kg, while animals receiving 2000 mg/kg showed signs of hypoactivity through 24 hours. Animals receiving higher doses (3000, 4000, and 5000 mg/kg) were generally hypoactive throughout the 48 hour observation period; ptosis was also observed.

2. SCE Assay: Based on the findings of the preliminary toxicity test, the doses selected for the SCE assay were 3000, 4000, and 5000 mg/kg. In agreement with the preliminary assay, all animals receiving tebuthiuron were hypoactive beginning one hour posttreatment and continuing throughout the study. One animal treated with 4000 mg/kg tebuthiuron was found dead 14 hours posttreatment; the cause of death was not reported. Cytotoxicity, as indicated by an increase in the percent of first division metaphases (M_1), was evident in all animals of the 5000-mg/kg group (Table 1). Although the report indicated that two of three animals in the low-dose group had an increased incidence of M_1 cells, the combined percentage was only slightly higher than the control. Results for the mid-dose females provided no evidence of cytotoxicity. No significant increase in the frequency of SCEs was seen in any of the animals treated with tebuthiuron. In contrast, a statistically significant ($p \leq 0.01$) increase was found in the animals treated with the positive control. From the overall findings, the authors concluded that tebuthiuron did not cause SCEs in the bone marrow of Chinese hamsters.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. Tebuthiuron was evaluated to a cytotoxic dose but failed to induce SCE. The response of the test system to the positive controls indicated that the assay was sufficiently sensitive to detect a genotoxic response. We, therefore, conclude that tebuthiuron did not induce SCE in this test system. However, the study is lacking in two aspects. First, only three female animals were tested per dose, instead of the recommended 5 animals per sex. No explanation was provided for using fewer animals and only one sex. Second, no mention was made of the methods used to assign the animals to treatment and control groups or whether the slides were coded prior to analysis to reduce bias.

TABLE 1. Results of the In Vivo Sister Chromatid Exchange Assay in Bone Marrow Cells of Female Chinese Hamsters Treated with Tebuthiuron

Substance	Dose	Number of Animals Analyzed[a]	Number of Metaphases Scored/Group	SCE/Metaphase (Group Mean ± S.D.)	$\%M_1$[b]
Vehicle Control					
10% Aqueous acacia	10 mL/kg	3	75	2.7±2.0	20
Positive Control					
Cyclophosphamide	50 mg/kg	3	75	16.7±7.8[c]	33[d]
Test Compound					
Tebuthiuron	3000 mg/kg	3	75	2.4±1.5	23
	4000 mg/kg	2[e]	50	2.8±1.6	14
	5000 mg/kg	3	75	2.3±1.7	35[d]

[a]All females.

[b]$\%M_1$ = the mean number of first division metaphases for each treatment group, based on analyzing 100 mitotic figures per animal.

[c]Significantly higher than control value at $p \leq 0.01$, using Student's t-test.

[d]Cytotoxic; distribution of metaphase figures shifted in favor of first division staining pattern.

[e]One animal died; results are from the two remaining animals.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated July 13, 1988.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-13; Appendix B, Protocol, CBI p. 19.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 9-13

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_____ A draft product label.

_____ The product confidential statement of formula.

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DOC 920078

FINAL

009434

DATA EVALUATION REPORT

TEBUTHIURON

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne T. Haber Ph.D. Date 4/7/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 4/7/92

QA/QC Manager Sharon Segal, Ph.D. Date 4/7/92

Contract Number: 68D10075
Work Assignment Number: 1-30
Clement Number: 91-95
Project Officer: James Scott

MUTAGENICITY STUDIES

EPA Reviewer: Ray Landolt Signature:
Review Section I, Toxicology Branch (II)/HED Date: 4/9/92
EPA Section Head: Yiannakis Ioannou, Ph.D. Signature:
Review Section I, Toxicology Branch (II)/HED Date: 4/9/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 366AA

MRID Number: 407509-01

TEST MATERIAL: Tebuthiuron

SYNONYMS: N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea

SPONSOR: Eli Lilly and Company, Greenfield, Indiana

STUDY NUMBER: 880510UDS0655 and 880517UDS0655

TESTING FACILITY: Eli Lilly and Company, Greenfield, Indiana

TITLE OF REPORT: The Effect of Tebuthiuron (EL-103, Compound 075503) on the Induction of DNA Synthesis in Primary Cultures of Adult Rat Hepatocytes.

AUTHORS: Negilski, D. S., Garriott, M.L., and Yount, D. J.

REPORT ISSUED: July 13, 1988

CONCLUSIONS-EXECUTIVE SUMMARY: Under the conditions of two independently performed unscheduled DNA synthesis (UDS) assays, at concentrations ranging from 300 to 800 µg/mL, tebuthiuron did not induce UDS in primary rat hepatocytes. Higher levels (≥900 µg/mL) were cytotoxic. Based on these findings, it was concluded that tebuthiuron was tested over an appropriate range of concentrations with appropriate controls and showed no evidence of UDS. However, fewer cells (20/culture) than recommended by Guideline (50 cells/culture) were counted. In addition, the report did not indicate whether slides were coded prior to analysis to reduce bias. Thus, the study does not fully satisfy Guideline requirements for genetic effects Category III, Other Mutagenic Mechanisms.

STUDY CLASSIFICATION: The study is currently unacceptable, but can be upgraded if the slides are still available and data from the analysis of at least 50 cells per culture are submitted for review. Slides should also be coded prior to analysis.

A. MATERIALS:

1. Test Material: Tebuthiuron

   Description: None provided
   Identification No: Compound 075503; Lot No.: 729AS7
   Purity: 99.1%
   Receipt date: Not reported
   Stability: Not reported
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: Neither storage conditions nor the frequency of dosing solution preparation were reported.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the livers of male Fischer 344 rats purchased from Charles River, Kingston, New York, and weighing ≈ 220 g.

3. Control Substances: DMSO at a final concentration of 1% was used as the solvent control; N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) at 1 to 20 µg/mL and 2-acetylaminofluorene (2-AAF) at 0.05 to 1 µg/mL were used as the positive controls.

4. Medium: WME: Williams' Medium E; WME+: Williams' Medium E with 10% fetal bovine serum and antibiotics.

5. Test Compound Concentrations Used: Two trials were performed with eight doses of the test material (300, 400, 500, 600, 700, 800, 900, and 1000 µg/mL).

B. STUDY DESIGN:

1. Cell Preparation:

a. Perfusion techniques: Rats livers were perfused in situ with Hank's balanced salt solution containing 0.5 mM EGTA and Hepes buffer, pH 7.2 to 7.4, for 1-2 minutes, and with WME containing 100 units/ml collagenase and Hepes buffer (pH 7.2) for 10-14 minutes. Cells were detached by combing in fresh WME-collagenase medium, filtered through gauze, washed, and centrifuged.

b. Hepatocyte harvest/culture preparation: Recovered cells were resuspended in WME+ and counted; cell viability was 85% and 88% for the initial and repeat experiments, respectively.

2. UDS Assay:

a. Treatment: Cells were plated at a density of 2.3×10^4 cells/cm^2 into multiwell culture dishes containing coverslips and treated with WME+ containing 1.0 mg/ml collagen. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 2.5-hour attachment period. Unattached cells were removed; viable cells were fed WME containing 10 µCi/mL [^{3}H] thymidine.

Single cultures were exposed to each of the selected test material doses or the positive controls (MNNG or 2-AAF), and four replicate cultures were treated with the solvent control (DMSO). Twenty hours posttreatment, the treated hepatocytes, attached to coverslips, were washed, exposed to 1% sodium citrate for 10 minutes, fixed in acetic acid:ethanol (1:3), dried, and mounted.

b. Preparation of autoradiographs/grain development: Slides were stained with Aceto-Orcein stain, coated with Kodak NTB-2 emulsion, dried for 7 days at 4°C in light-tight desiccated boxes, developed in Kodak D-19 developer, fixed, and counted. The report did not indicate whether slides were coded prior to analysis.

c. Grain counting: The nuclear grains of 20 morphologically unaltered cells that were judged to be representative of the UDS responsiveness of the cell population, and contained at least four grains, were counted for each test dose and the negative and positive controls. Cytoplasmic background counts were determined by counting three nuclear-sized areas adjacent to the nucleus. Net nuclear grain counts were determined by subtracting the mean cytoplasmic background count from the nuclear grain count. Grain counts were conducted for the highest test compound dose that did not produce pronounced cytotoxicity and for all lower concentrations.

4. Evaluation Criteria: The assay was considered positive if at least two successive concentrations of the compound produced mean net nuclear grain counts exceeding those of the control by three standard deviations of the control value.

5. Protocol: See Appendix B.

C. REPORTED RESULTS: Eight levels of tebuthiuron, ranging from 300 to 1000 µg/mL, in 100 µg/mL increments, were tested in two independently performed assays. Results from the two trials indicated that doses ≥800 µg/mL in trial 1 and ≥900 µg/mL in trial 2 were cytotoxic (Table 1). There was, however, no evidence of UDS at any of the non-cytotoxic concentrations of tebuthiuron tested. By contrast, the positive controls, MNNG and 2-AAF, induced marked increases in UDS. Based on these findings, the study author concluded that tebuthiuron was negative in the primary rat hepatocyte UDS assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. In two independently performed assays, tebuthiuron was evaluated to cytotoxic doses but failed to induce UDS. The response of the test system to the positive controls indicated that the assay was sufficiently sensitive to detect a mutagenic response. We, therefore, conclude that tebuthiuron did not induce UDS in this test system. However, the study is lacking in two aspects. First, only 20 cells per culture were counted, instead of the recommended 50 cells per culture. Even if the results from the two assays are combined, less than 50 cells per dose were counted. Second, no mention was made of coding the slides prior to analysis to reduce bias.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated July 13, 1988.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-13; Appendix B, Protocol, CBI p. 17.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assays with Tebuthiuron

Treatment	Dose/mL	Net Nuclear Grains[a] Initial Trial	Net Nuclear Grains[a] Repeat Trial
Solvent Control			
Dimethyl sulfoxide	1%	-3.0[b]	-3.9[b]
Positive Controls[c]			
N-methyl-N'-nitro-N-nitrosoguanidine	1 μg	26.4±10.4[d]	1.7±3.8
	5 μg	Toxic[e]	12.4±7.2[f]
2-Acetylaminofluorene	0.05 μg	17.9±5.5[f]	57.7±12.9[f]
Test Material			
Tebuthiuron	700 μg	-2.4±2.3[g]	-2.7±2.9
	800 μg[h]	Toxic	-2.9±2.7[g]

[a]Means and standard deviations from the count of 20 cells per culture.

[b]Four replicate means ± S.D. were presented; our reviewers calculated the average of the four means.

[c]MNNG was tested at 1, 5, 10, and 20 μg/mL; 2-AAF was tested at 0.05, 0.1, 0.5, and 1 μg/mL. Selected representative data are shown.

[d]Cytotoxic; surviving cells fulfill reporting laboratory's criterion for a positive response (i.e., net nuclear grain counts exceed those of the control by 3 SDs of the control value).

[e]Cytotoxicity preventing evaluation of UDS was observed at this and higher doses (10 and 20 μg/mL).

[f]Fulfills reporting laboratory's criteria for a positive response.

[g]Highest non-cytotoxic dose; findings for lower levels (300, 400, 500, and 600 μg in both trials) did not suggest a genotoxic effect.

[h]Doses ≥ 800 μg in initial trial (800, 900, and 1000 μg/mL), and doses of 900 and 1000 μg/mL in the repeat trial, were cytotoxic, preventing evaluation of UDS.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 9-13

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Pages 25 through 31 are not included in this copy.

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